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ON THE GENETIC CONTROL OF MUTABILITY IN MAIZE

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The *Dt* gene in maize is of more than usual interest because of its striking ability to increase the mutability of the recessive *a* allele which is normally highly stable.[1, 2] The locus of *a* is in chromosome 3; data were published in 1941 proving that *Dt* lies in chromosome 9, but its precise location in the linkage map was uncertain. The present paper is chiefly concerned with (1) the accurate location of *Dt*, (2) the regional distribution of crossing-over in the short arm of chromosome 9 and the location of the known mutant genes in this chromosome, and (3) a consideration of a paper by Goldschmidt[3] in which is offered an alternative explanation to the results interpreted by the author as the greatly increased mutability of the recessive *a* allele.

(1) According to the summary in Emerson, Beadle and Fraser,[4] the linear order and intervening map distances in chromosome 9 are as follows: yg_2 19 *C* 3 *sh* 15 *bp* 15 *wx* 12 v_1. The yg_2 locus is close to the end of the short arm with the other loci occupying more proximal positions. Four point back-cross data involving the yellow green-2 (yg_2), shrunken endosperm (*sh*), waxy endosperm (*wx*) and the *Dt* loci are presented in table 1. The *Dt* allele has no known effect other than to raise the mutation rate of the *a* allele; the classification for *Dt* and *dt* therefore rests upon the presence or absence of mutant areas showing the *A* phenotype. The data in table 1 place *Dt* seven cross-over units to the left of yg_2. Creighton and McClintock[5] and McClintock[6] have shown that yg_2 is very close to the terminal knob on the end of the short arm. Creighton found only 1.5 per cent crossing-over between yg_2 and the knob. The location of *Dt* seven units to the left of yg_2, which is only 1.5 units from the knob terminating the end of the chromosome, would seem an inconsistency. However, the cross-over value found by Creighton for the knob-yg_2 region is undoubtedly too low, since she worked with chromosomes 9, one of which possessed a large terminal knob while the other had a minute knob. It has been observed

that asynapsis frequently occurs with terminal heteromorphic knobs; this asynapsis should result in lower cross-over values in distal regions. Further, her low percentage of recombination was based on a relatively small population of 261 individuals, which would permit a large sampling error.

(2) The location of *Dt* at the extreme left end of the short arm of chromosome 9 is of interest in connection with McClintock's[6] recent investigations on mutations and deficiencies. The short arm of this chromosome at pachytene has about 20 chromomeres. Those chromomeres adjacent to the centromere are more deeply staining than the distal ones. In an elegant series of experiments she localized the yg_2 locus in a distal portion of the first chromomere. Joining this ultimate chromomere to the terminal knob is a thin, lightly staining chromatin thread to which she was able to assign the locus of a gene affecting chlorophyll development. Disregarding the heterochromatic terminal knob, the only part of chromosome 9 to the left of the yg_2 locus is the threadlike strand connecting the ulti-

TABLE 1

FOUR POINT BACK-CROSS LINKAGE DATA INVOLVING THE *Dt* GENE

$$\frac{Dt^{(1)}\ Yg^{(2)}\ Sh^{(3)}\ Wx}{dt\ yg\ sh\ wx} \times dt\ yg\ sh\ wx$$

(0)	(0)	(1)	(1)	(2)	(2)	(3)	(3)	(1–2)	(1–2)	(2–3)	(2–3)	(1– 3)
Dt	*dt*	*Dt*	*dt*	*Dt*	*dt*	*Dt*	*dt*	*Dt*	*dt*	*Dt*	*dt*	*dt*
Yg	*yg*	*yg*	*Yg*	*Yg*	*yg*	*Yg*	*yg*	*yg*	*Yg*	*Yg*	*yg*	*Yg*
Sh	*sh*	*sh*	*Sh*	*sh*	*Sh*	*Sh*	*sh*	*Sh*	*sh*	*sh*	*Sh*	*Sh*
Wx	*wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*Wx*	*wx*	*Wx*	*wx*	*wx*
880	672	88	99	225	309	251	196	3	4	12	13	3

Recombination percentages: *Dt-Yg* 7.2%
Yg-Sh 20.6%
Sh-Wx 17.2%

mate chromomere and the knob. *Dt* is seven cross-over units to the left of yg_2; unless it is situated within the terminal knob (a possibility which cannot be wholly dismissed even though the knobs are believed to be composed of genetically inert heterochromatin), it must lie somewhere on the threadlike strand. The total length of the genetic map of the short arm of chromosome 9 is approximately 60 units. Cytologically this short arm has approximately 20 chromomeres. Seven of the 60 cross-over units lie to the left of the ultimate chromomere. This is a much greater proportion than should occur if the amount of crossing-over per unit of length was of the same order along the short arm and suggests that a disproportionately high amount occurs in the distal portion of the short arm of chromosome 9.

It is perhaps significant that the *Dt* gene, which exerts such a profound influence on the mutability of another gene, is located near to or in the heterochromatic knob terminating the end of the short arm of chromo-

some 9. The mechanisms through which the *Dt* gene alters the mutability of *a* are unknown, but on certain hypotheses the effect of *Dt* would be modified if it were interstitially rather than terminally situated.

The location of *Dt* in the short arm of chromosome 9 is in no way surprising, but it might be pertinent to mention the distribution of mutant loci in this chromosome. The long arm of 9 is approximately twice the length of the short arm, yet of the thirty-odd mutant genes belonging to this chromosome not one has been located definitely in the long arm, although virescent-1 may fall here.[7] The failure to find a proportionate share of mutant loci in the long arm may reasonably be attributed to the existence of duplications.[8] These duplications may exist within the long arm of 9 itself or may be found in certain of the other chromosomes. A similar condition possibly exists for chromosome 5. The two arms are approximately equal in length, yet the great majority of the mutant genes have been found to lie in the slightly longer arm. With the exception of chromosome 8, which is as yet sparsely populated with mutant genes, such a marked localization of genes to restricted portions of the chromosome as occurs in chromosomes 9 and 5 does not appear to hold for the remaining chromosomes, although more information on this point is needed. That duplications do exist in maize is attested by the occurrence of occasional bivalents at M I in haploid plants. The existence of duplications is of interest in connection with the problem of the origin of maize. It has been held that maize is an ancient amphidiploid resulting from the cross of two five-chromosome species. The notion that five is the basic number rather than ten has been entertained ever since two close relatives, Coix and Sorghum, were found to have species with a haploid set of five chromosomes.

(3) In a recent paper, Goldschmidt[3] described a situation in *Drosophila melanogaster* in which factor interaction gives a variety of phenotypes resembling in some degree at least the array of types found by Demerec[9] for the unstable miniature alleles of *Drosophila virilis*. The $bran^{dp}$ allele of the arc locus in chromosome 2 has no phenotypic effect unless the recessive allele, svr^{poi}, at the silver locus in the X chromosome is present. Flies lacking the $bran^{dp}$ allele but homozygous for svr^{poi} have pointed wings, while flies with $bran^{dp}$ and svr^{poi} exhibit a considerable diversity of phenotypes. The majority have pointed wings like those of svr^{poi}, the remainder have one wing pointed and one truncated or else have "transitions from a pointed to a truncated wing in all conditions of asymmetry down to a symmetrical, truncated (dumpy-like) wing." Goldschmidt believes the $bran^{dp}$ allele to be one which acts near the threshold between the pointed wing and the dumpy wing phenotypes and holds that the range of phenotypes may be satisfactorily accounted for by epistasis near the threshold value and by consideration of the develop-

mental physiology of the wing. Goldschmidt states that the phenotypes found by Demerec for the unstable *mt-c* allele resemble those found in *bran*^{dp} *svr*^{poi} flies and suggests that the unstable gene hypothesis be abandoned. He argues that the *mt-c* allele is not unstable but is influenced by another gene, similar to *bran*^{dp}, which has no effect alone but which interacts with *mt* in the direction of normal wing development. He reports another arc allele, *bran*^{r}, which gives an array of phenotypes resembling those produced by the unstable *mt-a* allele in *virilis*.

In the belief that he has satisfactorily accounted for the variety of phenotypes found for the unstable miniature alleles in *virilis* by a hypothesis which does not require the high mutation rate postulated by Demerec, he next considers variegation in plants. Reviewing the *a-Dt* situation in maize reported by the author, he suggests that the variegation found here can also be accounted for by his hypothesis of factor interaction, epistasis and threshold values. Specifically he cites the *a* allele as comparable to *svr*^{poi}, while *Dt* would parallel *bran*^{dp}. To account for mutations of *a* to *A* in sporogenous tissue, giving rise in many cases to anthers with half of the pollen carrying the *a* allele and the other half possessing the dominant *A* allele (derived by mutation), he assumes that the mutations of *a* to *A*, yielding self-colored seeds and plants, are not changes of *a* to *A*, but are due to the introduction through outcrossing of a new allele of *Dt* equal to *A* in its ability to produce anthocyanin in the aleurone, plant and pericarp tissues. This new allele of *Dt* is therefore comparable to the *bran*^{r} allele. Goldschmidt states that no published results disprove his hypothesis. In this he errs, because several decisive observations which negate his interpretation have been published. First, the location of the *a* allele is in chromosome 3 while *Dt* is in chromosome 9. The newly arisen mutations of *a* to *A* show the same linkage relations with genes in chromosome 3 as do standard *A* alleles. On Goldschmidt's hypothesis the new color-producing genes should exhibit linkage with loci in chromosome 9 since *Dt* lies in that chromosome. Second, when a mutation of *a* to *A* occurs in a cell of *a a Dt Dt* constitution, the genotype of that cell and its descendents is *A a Dt Dt*. On his hypothesis the constitution should be *a a Dt Dt*^{A} (where *Dt*^{A} represents the new *Dt* allele equal to *A* in its ability to form anthocyanins). Third, Goldschmidt postulates the existence of a new allele of *Dt* which produces the same phenotypic effect as does *A*. At least five different alleles at the *a* locus have been obtained by mutation of *a* in the presence of *Dt*. On his hypothesis there must be five different *Dt* alleles, yet the *Dt* gene comes from a single source. They could arise only by mutation, so his hypothesis would merely shift the mutability from the *a* allele to the *Dt* allele, and he would still have to contend with the high mutability of the *Dt* allele.

Interesting though Goldschmidt's studies are on factor interaction in

Drosophila melanogaster, and however closely their phenotypic expression may simulate those produced by unstable genes, they can hardly be considered as negating the validity of the mutable gene hypothesis.

Summary.—Data are presented showing that the *Dt* allele, which profoundly affects the mutability of the recessive *a* allele, is located at the end of the short arm of chromosome 9. The regional distribution of crossing-over in the short arm of chromosome 9 and the location of the known mutant genes in chromosome 9 are considered. An examination of the published data on the genetic control of the mutation rate of the recessive *a* allele reveals that it cannot be accounted for in terms of factor interaction, epistasis and threshold values as Goldschmidt suggests.

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*MAINTENANCE AND INCREASE OF A GENETIC CHARACTER BY A SUBSTRATE-CYTOPLASMIC INTERACTION IN THE ABSENCE OF THE SPECIFIC GENE**

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Introduction.—Our previous studies[1,2] on the production of galactozymase by *Saccharomyces cerevisiae* revealed that in certain diploid strains its appearance in the cell was effected by a direct interaction between the cytoplasm and galactose. In certain haploid strains, mutation was apparently required before adaptation to galactose fermentation occurred. Study[3] of other yeast types supported the view that mutation in the haplophase was one of the mechanisms of adaptation. Thus, it was possible to adapt the normally diploid *Schizosaccharomyces pombe* (which had previously been reported[4] as unadaptable) to galactose fermentation by inoculating a galactose medium with a heavy suspension of haploid spores.

Information on the relation of specific genes to adaptation was pro-

vided by an analysis of the genetics of adaptation to melibiose fermentation.[6] In this investigation progenies of a hybrid between *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* were used. *S. carlsbergensis* and all its haploid segregants can adapt to melibiose fermentation; *S. cerevisiae* and its haploid progeny cannot. Data obtained from 175 progenies of the interspecific and of related hybrids were consistent with the view that *S. carlsbergensis* is homozygous for two pairs of dominant genes, any one of which is capable of producing the adaptive enzyme.

The experiments with adaptation to both galactose and melibiose led to the conclusion that the gene initiates the synthesis of the adaptive enzyme, providing the specific substrate is present. The data showed that, within wide limits, the amount of adaptive enzyme in the cytoplasm depends on an interaction between the cytoplasm and the substrate. These results raised the following questions:

1. If synthesis has been initiated, can the substrate-cytoplasmic interaction maintain the enzyme indefinitely in the cytoplasm in the absence of the specific gene?
2. If some enzyme is present, can synthesis of additional enzyme occur in the absence of the specific gene necessary to initiate its synthesis?

The opportunity for answering these questions was provided by analysis of certain progenies of the *S. cerevisiae* by *S. carlsbergensis* pedigree.[5] Both questions were answered in the affirmative. Not only can the cells increase their ability to ferment melibiose in the absence of the gene but this character has been maintained in these cells for over 1000 generations.

Methods.—A. Matings and Isolation of Haploid Segregants: The general methods for inducing ascospore formation, their dissection and subsequent treatment for the production of hybrids and isolation of haploid strains have been described elsewhere.[6, 7, 8, 9]. For purpose of later discussion, the steps involved in the formation of a hybrid and the analysis of its haploid segregants are listed:

1. Mating by mixture of two haploid strains of proper mating types.
2. Induction of sporulation.
3. Dissection of 4-spored asci.
4. Planting of the four spores separately on agar.
5. Testing of the resulting clones for the character.

B. Test for Ability to Ferment Melibiose: All clones were characterized according to their ability to ferment melibiose by the standard inverted tube technique. These tests were run in duplicate and held for a period of more than four weeks before being discarded as negative. All these tests were checked by Warburg manometric methods, in which the rate of CO_2 evolution in nitrogen by a suspension of the cells in $M/15$ KH_2PO_4 was measured after the melibiose had been added from a sidearm. The nitrogen

used to displace the air in measurements on anaerobic CO_2 production was passed over hot copper to remove any traces of oxygen. All measurements were taken at 30.2°C. and the vessels were shaken at a rate of 100 oscillations per minute over a 7-cm. arc. The basic medium and carbohydrate, as well as their treatment and preparation, were the same as those used previously.[5]

Experimental.—In the previous[5] experiments on adaptability to melibiose fermentation, the cells came into contact with melibiose for the first time in the test for adaptability, and the first four steps (see *Methods*) were performed without the addition of melibiose. In the present experiments the effect of performing all five steps in the presence of melibiose (3%) was tested. The reason for this procedure lies in the fact that a cellular adaptive enzyme is stabilized by keeping the cells in contact with the specific substrate. A hybrid was obtained by mating an adaptable haplophase clone carrying a single *S. carlsbergensis* gene controlling adaptation, to a haplophase clone of *S. cerevisiae* which carried the allele controlling non-adaptability. These heterozygous diploid hybrids are all adaptable, for the mel + gene is dominant. Each 4-spored ascus from a heterozygous diploid cell yields two adaptable and two unadaptable haplophase cultures.

TABLE 1

EFFECT OF MELIBIOSE ON PHENOTYPIC CHARACTERS OF SEGREGANTS FROM DIPLOIDE FORMED BY MATINGS IN ITS PRESENCE AND ABSENCE. + INDICATES ABILITY TO FERMENT MELIBIOSE, − INABILITY. ALL SPORES COME FROM A (+ × −) CROSS. (SEE TEXT FOR FURTHER DETAILS)

MATING, SPORULATION AND PLANTING IN PRESENCE OF MELIBIOSE					MATING, SPORULATION AND PLANTING IN ABSENCE OF MELIBIOSE				
ASCUS NO.	SPORES A	B	C	D	ASCUS NO.	SPORES A	B	C	D
1	+	+	+	+	8	+	+	−	−
2	+	+	+	+	9	−	+	−	+
3	+	+	+	+	10	+	+	−	−
4	+	+	+	+	11	+	+	−	−
5	+	+	+	+	12	−	+	−	+
6	+	+	+	+	13	−	−	+	+
7	+	+	−	−	14	+	−	−	+
					15	+	+	−	−
					16	+	−	+	−
					17	−	−	+	+

Table 1 summarizes the results of these experiments. Asci 1–15 originated from mating a pair of mel+/mel− haploids while 16 and 17 originated from mating a different pair of mel+/mel− haploids. Melibiose was present in the substrate in all five steps in the formation and dissection of asci 1–7, inclusive. Asci 10–17, inclusive, were formed in the usual way, without melibiose. In handling asci 8 and 9 the agar in which the spores were planted contained melibiose (step 4, *Methods*) although melibiose was absent in the first 3 steps.

It is evident from table 1 that all asci formed in the complete absence of melibiose give the typical 1:1 ratio characteristic of a heterozygous hybrid segregating a single pair of genes. These results agree with those reported previously[5] on equivalent crosses. On the other hand, with the exception of ascus No. 7, identical heterozygotes treated with melibiose yielded four adaptable spores from each ascus.

The results obtained without melibiose prove that only two spores of each tetrad in asci 1–6, inclusive, contain the specific gene responsible for adaptation to melibiose fermentation. Despite this, all four spores from these tetrads produced haplophase cultures which fermented melibiose.

Since all steps were carried out in the presence of melibiose, selection of adaptable mutants from haploids originally unable to ferment melibiose might have occurred. Step 4 particularly is open to such criticism. However, several specific facts rule out this possibility: (1) During the testing of many segregants from *S. cerevisiae*, all of which are negative, no mutation to an adaptable type has been observed whether melibiose was present or not. (2) The same is true of negative haploids from heterozygous hybrids. No mutations to the adaptable type have been seen among these no matter how often they have been transferred through melibiose media. (3) Asci 8 and 9 whose segregants were planted on melibiose, yielded the standard 1:1 ratio.

More conclusive evidence on this point was obtained by the following experiments. Presumably, the cultures from two spores of each tetrad (from the first six asci) were able to ferment melibiose only due to the presence of the adaptive enzyme in the cytoplasm. Consequently, it is to be expected that removal of the melibiose would lead not only to the disappearance of fermentability in all cases, but to an eventual loss of readaptability in two of every four cultures arising from each of the first six asci. To exclude the complication of mutations away from adaptability[3] only non-dividing cultures suspended in $M/15$ KH_2PO_4 were used. The 24 adapted haplophase cultures originating from the first six asci were all grown in the basic culture medium containing melibiose as the sole carbohydrate source. Using sterile precautions the cells were washed free of the medium with $M/15$ KH_2PO_4 and resuspended in sterile $M/15$ KH_2PO_4 to make suspensions containing approximately 8 mg. dry weight of yeast per cc. Each suspension was divided into two equal parts, and sufficient melibiose was added to one portion to make a 4% solution. This was used as a control to test the stability of the enzyme in the presence of the substrate under the experimental conditions. The other portion of each suspension did not receive any melibiose. The flasks were then shaken continuously at 28°C. and cells were removed at intervals to test them for the ability to ferment melibiose.

None of the control suspensions containing melibiose showed loss of the

ability to ferment this sugar within the experimental period. On the other hand, all of the suspensions without substrate showed decreases within 24 hrs. in their rates of anaerobic CO_2 evolution when melibiose was added. In varying periods of time, ranging from 7–20 days, all of these suspensions lost the ability to evolve significant amounts of CO_2 anaerobically on immediate contact with melibiose. Twenty-four hours after a suspension showed insignificant rates of CO_2 evolution in the presence of melibiose (i.e., $Q^N_{CO_2}$ values of less than unity) a sample was removed and incubated with melibiose aerobically at 30.2°C. to test for readaptability. At the same time its ability to ferment glucose was also examined. This was done to avoid testing cells whose physiological condition was seriously impaired by the long vigorous shaking in the phosphate buffer without substrate. With cells unable to ferment glucose the inability to readapt to melibiose would be difficult to interpret. Three suspensions of the original 24 were eliminated on this basis. The data on the asci producing four testable segregants are given in table 2. The removal of melibiose and its stabiliz-

TABLE 2

READAPTABILITY OF SPORES OBTAINED BY MATINGS IN PRESENCE OF MELIBIOSE AFTER HAVING LOST ALL ADAPTIVE ENZYMES. + INDICATES READAPTABILITY, − INABILITY

ASCUS NO.	SPORES A	B	C	D
1	+	−	+	−
2	−	−	+	+
4	+	+	−	−
6	+	+	−	−

ing influence leads to the reappearance of the expected Mendelian ratios. These results give further support to the view that only two spores in each of these tetrads carried the mel+ gene.

In addition, data collected at the same time show that synthesis of additional enzyme can occur in the absence of the specific gene necessary to initiate the synthesis. After allowing all suspensions to fall to low $Q^N_{CO_2}$ values (between 1.8 and 10.1), portions were removed and incubated with melibiose and regeneration of activity followed at intervals by measuring the rate of anaerobic CO_2 evolution. The results on those haploid segregants which subsequently lost the ability to adapt are recorded in table 3. It is seen that in all cases, marked increases in activity were obtained. It may be noted here without going into detail that no significant difference in the rate of increase in enzyme activity could be established between the cells carrying the non-fermenting allele (mel−) and those which possessed the mel+ gene. As far as can be determined it appears that the rate of enzyme regeneration is critically determined by the enzyme and substrate content of the cytoplasm at the outset of the incubation period.

All the strains listed in table 3 were carried in standard media with melibiose and were tested at weekly intervals. At the end of three months they could all ferment melibiose at a rate equal to or greater than the original rate. This period is equivalent to over 1000 cell generations. It is thus evident that melibiose can maintain the enzyme in the cytoplasm of these cells while active division is going on for long periods of time.

Discussion.—These results appear to indicate that melibiose can obscure the nuclear hereditary mechanism because it is the specific substrate for a cytoplasmic enzyme whose activity level depends critically on the amount of substrate available. The fact that adaptability was lost on the removal of substrate proves that a directed mutation under the influence of melibiose is not involved.

An explanation of the results obtained, in terms of enzymes and their specific substrates, may be briefly summarized as follows: By performing the mating in the presence of melibiose, the cytoplasm of the haploid gamete carrying the mel+ gene is packed with the melibiose fermenting

TABLE 3

$Q^{N}_{CO_2}$ VALUES AFTER AEROBIC INCUBATION WITH MELIBIOSE OF STRAINS WHICH EVENTUALLY LOST THEIR ABILITY TO ADAPT

STRAIN	HOURS IN CONTACT WITH MELIBIOSE			
	0	12	24	48
1B	5.1	40	96	123
1D	2.4	26	109	114
2A	10.1	39	86	136
2B	6.3	46	73	101
4C	5.0	69	160	170
4D	4.2	29	91	134
6C	1.8	34	84	141
6D	4.8	42	121	130

enzyme. Since both copulating haploids contribute cytoplasm equally to the zygote, it contains the enzyme. Because sporulation occurs in the presence of melibiose, the enzyme molecules are stabilized and possibly increased in amount, since the pre-sporulation period is characterized by growth and considerable storage. Each of the four haploid segregants derives its cytoplasm from the diploid hybrid and it follows that each will have enzyme molecules in its cytoplasm no matter what its genetic constitution. Finally, the enzyme molecules are stabilized even in the descendents of the spores which do not have the mel+ gene by keeping the cells in contact with melibiose. From this point of view, both maintenance and increase of the enzyme activity can be effected by a direct cytoplasmic interaction with the substrate in the absence of the specific gene. Therefore, as far as melibiozymase is concerned the function of the mel+ gene would be limited to the initiation of its synthesis.

The experiments reported here do not rule out the possibility of a more direct genic involvement than is implied in the explanation proposed above. Thus, e.g., the data could be satisfied by assuming the existence of a third gene in the carlsbergensis genome in addition to the two mel+ genes already described. This third gene, however, would have to possess the following properties or their equivalents:

(*a*) It can be stimulated to functional activity in the presence of either one of the mel+ genes and the substrate, but not by melibiose alone.

(*b*) Once activated it can remain functional in the absence of both mel+ genes, providing melibiose is present.

(*c*) It becomes irreversibly deactivated if melibiose is removed and all the melibiozymase exhausted from the cytoplasm.

Various modifications of this type of genic theory may be easily constructed. However, on the basis of the available evidence, the most plausible explanation of the data which can be advanced at present is in terms of substrate stabilized enzymes and the effect of their specific substrates on their maintenance.

The fact that enzymes in the cytoplasm can be influenced by their specific substrates provides a mechanism by which some of the phenomena of cytoplasmic inheritance can be explained. Tronsitory cytoplasmic effects could result from residual enzyme molecules which are not stabilized by the proper substrate. Lindegren[10] explains the degeneration of hybrids showing heterosis on this basis. The importance of cytoplasmic units in inheritance has been emphasized by the work of Winge and Laustsen,[11] Sonneborn[12] and Rhoades[13] among others and the speculations of Wright[14] and Darlington.[15] The adaptive enzyme, melibiozymase is a self-perpetuating cytoplasmic entity but differs from Darlington's "plasmagene" in being gene initiated and substrate stabilized.

Since substrate can evoke enzymatic activity previously non-detectable, it is conceivable that this phenomenon may also play a role in cellular differentiation.

Summary.—The effect of melibiose on the inheritance of the ability to ferment this sugar has been studied. In the absence of melibiose, a 1:1 ratio of fermentors to non-fermentors is exhibited by the four haploid segregants from an ascus. This is typical of a heterozygous diploid and agrees with the known genetic background of the hybrid employed. When the segregation occurs in the presence of melibiose all four segregants can ferment the sugar. All four haploids and the clones derived from them can maintain this ability indefinitely if kept in contact with the substrate. When, however, the melibiose is removed, only two out of the four can readapt to its fermentation. These data are explained in terms of adaptive enzymes and their stabilization by their specific substrates in the absence

of the gene; transfer of the character from one cell generation to the next is apparently effected by the enzyme molecules contained in the cytoplasm.

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ON SURFACE AREA

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1. We shall be concerned in this note with Fréchet surfaces of the type of the 2-cell.[7,8] Such a surface S admits of a representation of the form $S: x = x(u, v), y = y(u, v), z = z(u, v), (u, v) \in Q$, where Q is the unit square $0 \leqq u \leqq 1, 0 \leqq v \leqq 1$, and $x(u, v), y(u, v), z(u, v)$ are continuous in Q. The Lebesgue area of S will be denoted[8] by $A(S)$. We introduce a quantity $a(S)$, to be termed the lower area of S, using conceptions in the theory of continuous transformations in the plane.[3,6] These conceptions will be briefly reviewed presently.

2. Let D be a bounded domain (connected open set) in the uv plane. A bounded continuous transformation T, from D into a $\xi\eta$ plane, is given in the form $T: \xi = \xi(u, v), \eta = \eta(u, v), (u, v) \in D$, where $\xi(u, v), \eta(u, v)$ are bounded continuous functions in D. It is not assumed that T is bi-unique. With each point (ξ, η) there is associated[3] its essential multiplicity $\kappa(\xi, \eta,$

T, D) with respect to T and D. For each point (ξ, η) we consider the set $T^{-1}(\xi, \eta)$ in D. A maximal model continuum for (ξ, η) under T in D is a compact component of $T^{-1}(\xi, \eta)$. Such a continuum may or may not be essential.[3] We define a subset E^* of D as follows: E^* is the sum of all the essential maximal model continua, under T in D, corresponding to all the points (ξ, η) of the $\xi\eta$ plane. The set E^* depends upon both T and D. Let $\bar{D}$ be a subdomain of D. We denote by $\varphi(\bar{D})$ the measure of the set of those points (ξ, η) where $\kappa(\xi, \eta, T, \bar{D}) \pm 0$. In particular, $\varphi(\bar{D})$ is thus defined for rectangles in D. The derivative[3] of this rectangle function, if it exists at a point (u, v), will be denoted by $D_e(u, v)$. Here and in the sequel, a subscript e refers to the fact that the quantity involved is defined in terms of the essential multiplicity κ. We define further a subset N of D as follows. A point $(u_0, v_0) \epsilon D$ belongs to N if and only if the following conditions hold: (i) $(u_0, v_0) \epsilon E^*$; (ii) there exists an open set G such that $(u_0, v_0) \epsilon GD$, and the set $G - (u_0, v_0)$ contains no point of any essential maximal model continuum of the point $T(u_0, v_0)$. It follows that there exist simple closed curves C of arbitrarily small diameter, such that (u_0, v_0) is interior to C and the topological index of the point $T(u_0, v_0)$ with respect to the image of C under T is different from zero.[3] It follows further that this index is independent of C if the diameter of C is sufficiently small. We denote this index by $i_e(u_0, v_0)$. For points (u, v) not in N, we put $i_e(u, v) = 0$. If $D_e(u, v)$ exists at a point (u, v), then the quantity $J_e(u, v) = i_e(u, v)D_e(u, v)$ will be termed the essential generalized Jacobian.[3] By an oriented rectangle r we mean a rectangle with sides parallel to the u and v axes, respectively. r° denotes the interior of r. If the rectangle function $|T(r^\circ E^*)|$ is absolutely continuous[3] in D, then the transformation T will be said to be eAC in D (essentially absolutely continuous in D). If the essential multiplicity function $\kappa(\xi, \eta, T, D)$ is summable, then T will be said to be eBV in D (of essential bounded variation in D).[3,6]

3. Given a surface S as in 1, we introduce the three transformations (projections upon the coordinate planes):

$$T_x: y = y(u, v),\ z = z(u, v),\ (u, v) \epsilon Q^0,$$
$$T_y: z = z(u, v),\ x = x(u, v),\ (u, v) \epsilon Q^0,$$
$$T_z: x = x(u, v),\ y = y(u, v),\ (u, v) \epsilon Q^0.$$

The given representation of S will be termed[6] eBV if T_x, T_y, T_z are eBV in Q^0, and eAC if T_x, T_y, T_z are eAC in Q^0. The essential generalized Jacobians of T_x, T_y, T_z will be denoted by X_e, Y_e, Z_e, respectively. We shall denote the arithmetic square root of $X_e^2 + Y_e^2 + Z_e^2$ by W_e. If W_e exists almost everywhere in D and is summable in Q^0, then its integral over Q^0 will be denoted by I_e. If the first partial derivatives of $x(u, v)$, $y(u, v)$, $z(u, v)$ happen to exist at a point (u, v), then X, Y, Z will denote the Jacobians of T_x, T_y, T_z in the usual sense. W will then denote the arithmetic square

root of $X^2 + Y^2 + Z^2$. If W exists almost everywhere in Q^0 and is summable in Q^0, then its integral over Q^0 will be denoted by I. If $\bar{D}$ is a domain in Q^0, then we shall denote by $g_x(\bar{D})$ the integral of $\kappa(y, z, t_x, \bar{D})$ if this integral exists in the Lebesgue sense; otherwise we put $g_x(\bar{D}) = +\infty$. The quantities $g_y(\bar{D})$, $g_z(\bar{D})$ are defined in a similar manner. Finally, we denote by $g(\bar{D})$ the arithmetic square root of $g_x(\bar{D})^2 + g_y(\bar{D})^2 + g_z(\bar{D})^2$. Let now $\bar{D}_1, \bar{D}_2, \ldots$ be any finite or infinite system of disjoint domains in D. The least upper bound of the summation $g(\bar{D}_1) + g(\bar{D}_2) + \ldots$, for all possible systems $\bar{D}_1, \bar{D}_2, \ldots$, is defined as the lower area $a(S)$ of S.

4. If we restrict, in the definition of $a(S)$, the domains $\bar{D}_1, \bar{D}_2, \ldots$ by permitting only simply connected domains, then we obtain a quantity that has been studied by Reichelderfer,[6] and has been called by him the essential area of S. Clearly $a(S)$ is an upward revision of the essential area, which itself is an upward revision of other and analogously defined lower areas. The lower area $a(S)$, defined above, is always less than or equal to the Lebesgue area $A(S)$. Generally speaking, most substantial results concerning the Lebesgue area were achieved in cases where it could be shown that some kind of a lower area agreed, under the special circumstances involved, with $A(S)$. The lower area $a(S)$, introduced above, is the largest lower area studied so far, and thus it may be expected that it will agree with $A(S)$ under more general conditions. On the other hand, it is conceivable that in the process of upward revision we lose desirable properties as compared with previously used smaller lower areas. The outcome, as it appears at this time, is summarized in the following statements.

5. THEOREM. *Given S as in 1, suppose that $A(S) < +\infty$. Then the given representation of S is eBV in Q^0, the essential generalized Jacobians X_e, Y_e, Z_e exist almost everywhere in Q^0, and $I_e \leqq A(S)$ (cf. 3). The sign of equality holds if and only if the given representation is eAC in Q^0.*

6. THEOREM. *Given S as in 1, suppose that $A(S) < +\infty$, and suppose also that the first partial derivatives of $x(u, v)$, $y(u, v)$, $z(u, v)$ exist almost everywhere in Q^0. Then $I \leqq A(S)$ (cf. 3), and the sign of equality holds if and only if the given representation of S is eAC in Q^0.*

7. THEOREM. *Given S as in 1, suppose that $A(S) < +\infty$. Let $R_1, \ldots, R_m$ be a finite system of simply connected Jordan regions, without common interior points, whose sum is Q. Let $C_1, \ldots, C_m$ be the boundary curves of $R_1, \ldots, R_m$, and let $C_1^*, \ldots, C_m^*$ be the point-sets, in xyz space, that correspond to $C_1, \ldots, C_m$ by means of the given representation of S. Finally, let S_i be the surface determined by the given representation of S if (u, v) is restricted to R_i, $i = 1, 2, \ldots, m$. If the projections, upon the coordinate planes, of $C_1^*, \ldots, C_m^*$ are all of (planar) measure zero, then $A(S) = (AS_1) + \ldots + A(S_m)$.*

8. THEOREM. *Given S as in 1, we have always $a(S) \leqq A(S)$. If either $A(S) < +\infty$ or $a(S) = 0$, then $a(S) = A(S)$. If S admits of a represen-*

tation of the form $z = f(x, y)$, where $f(x, y)$ is single-valued and continuous in a simply connected Jordan region, then always $a(S) = A(S)$.

9. THEOREM. *Given S as in 1, let p denote a plane through the origin in xyz space. Let ξ, η denote Cartesian coordinates in p and let $\kappa_p(\xi, \eta, Q^0)$ denote the essential multiplicity function associated with the orthogonal projection of S upon p. Let us define α_p as the integral of $\kappa_p(\xi, \eta, Q^0)$ if this integral exists, and let us put $\alpha_p = +\infty$ otherwise. Let P be a point on the unit sphere $x^2 + y^2 + z^2 = 1$, and let us put $\alpha(P) = \alpha_p$, where p is the plane through the origin that is perpendicular to the radius of the unit sphere that joins P to the origin. We have then the following generalization of a well-known theorem of Cauchy.*

If $A(S) < +\infty$, then $\alpha(P)$ is summable on the surface of the unit sphere, and its integral mean value (taken over the surface of the unit sphere) is equal to $A(S)/2$.

10. As regards the proofs, the decisive points may be indicated as follows. Due to the upward revision mentioned above, the lower area $a(S)$ can be shown to be equal to $A(S)$ in the cases described in the theorem in section 8. On the other hand, it can be shown that in spite of the upward revision the lower area $a(S)$ remains sufficiently close to previously studied lower areas to enable one to extend, after proper modifications, previously developed methods[1, 3, 4, 6] to $a(S)$. A few further remarks should be made concerning the theorem in 8. The second and third alternatives considered there are rather immediate consequences of previous results.[5, 3, 6] The proof of the fact that $a(S) = A(S)$ if $A(S) < +\infty$ is based essentially upon the following results. The given representation of S, interpreted as a transformation from Q into xyz space, gives rise to a monotone-light factorization, to a corresponding middle-space M, and finally to partial mappings corresponding to the proper cyclic elements of M.[1, 2, 8] Let $S_1, S_2, \ldots$ be the surfaces determined by these partial mappings (if M is a dendrite, then these surfaces are missing, and the summations in the next statement are understood to be equal to zero). Then we have the formulas $A(S) = A(S_1) + A(S_2) + \ldots$, $a(S) = a(S_1) + a(S_2) + \ldots$. To secure the second one of these formulas, the upward revision of the lower area, mentioned above, seems to be indispensable. The first of these formulas was first studied by Morrey.[1, 2] His proof was based on a characterization theorem for representations defining the same surface S. Youngs found that this characterization theorem was false, derived an improved characterization theorem for the case of surfaces of the type of the 2-sphere, and proved[8] (for the 2-sphere case) the formula $A(S) = A(S_1) + A(S_2) + \ldots$. It seems, however, that even this improved characterization theorem of Youngs fails to hold for surfaces of the type of the 2-cell. However, the decisive formula $A(S) = A(S_1) + A(S_2) + \ldots$ can be established independently of the difficult topological issues suggested by the preceding observations.

11. It is entirely conceivable that the lower area $a(S)$, used in this study, is always equal to the essential area.[6] A more fundamental issue arises in connection with the theorem in 8. In view of that theorem, one may be excused for surmising that always $a(S) = A(S)$. If true, this fact would have far-reaching applications.

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[2] Morrey, C. B., "The Topology of (Path) Surfaces," *ibid.*, **57**, 17–50 (1935).

[3] Rado, T., and Reichelderfer, P., "A Theory of Absolutely Continuous Transformations in the Plane," *Trans. Am. Math. Soc.*, **49**, 258–307 (1941).

[4] Rado, T., and Reichelderfer, P., "Convergence in Length and Convergence in Area," *Duke Math. Jour.*, **9**, 527–565 (1942).

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[6] Reichelderfer, P., "On Bounded Variation and Absolute Continuity for Parametric Representations of Continuous Surfaces," *Trans. Am. Math. Soc.*, **53**, 251–291 (1943).

[7] Youngs, J. W. T., "Curves and Surfaces," *Am. Math. Monthly*, **51**, 1–11 (1944).

[8] Youngs, J. W. T., "The Topological Theory of Fréchet Surfaces," *Ann. Math*, **45** 753–785 (1944).